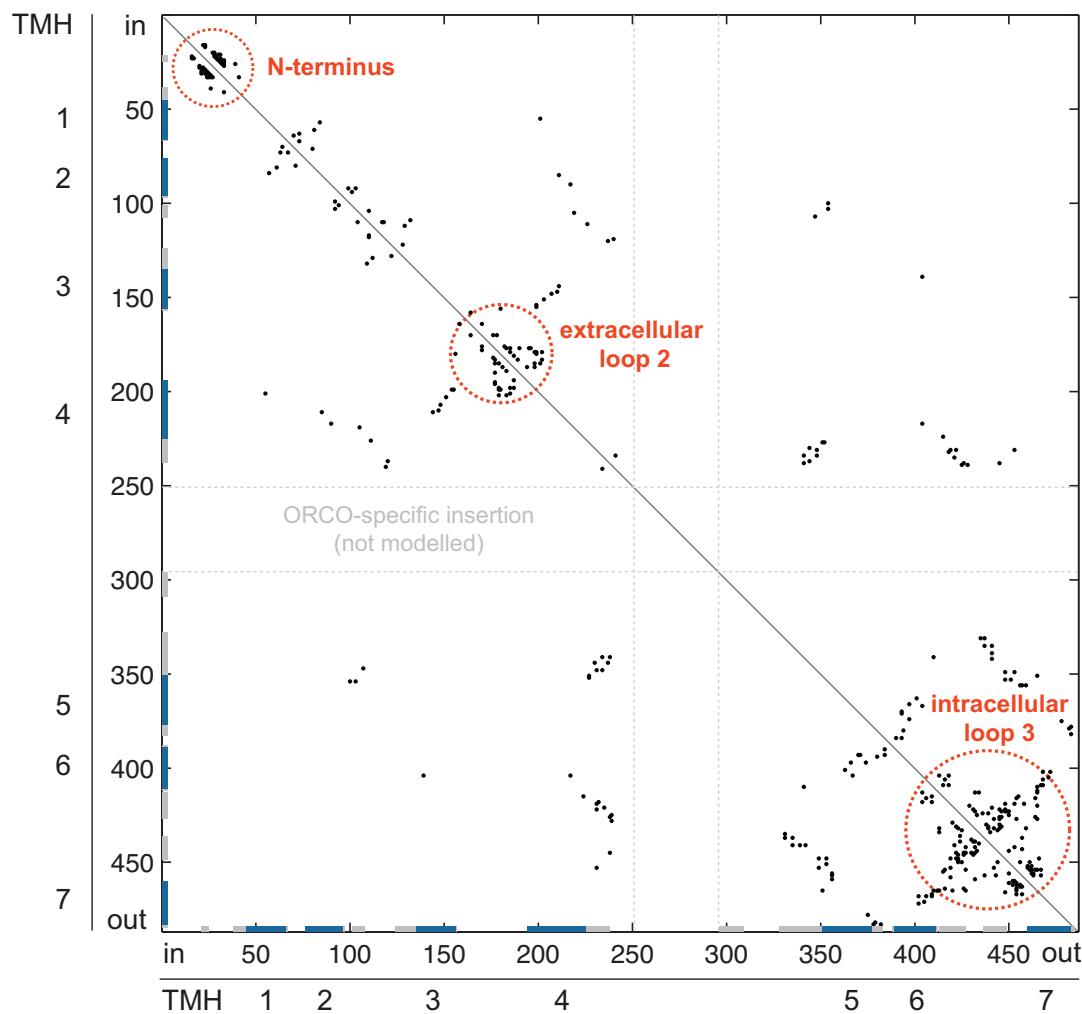


Amino acid coevolution reveals three-dimensional structure and functional domains of insect odorant receptors

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Supplementary Information

D. melanogaster ORCO, top 200 ECs



Supplementary Figure 1 | Contact map representation of the top 200 predicted ECs for *D. melanogaster* ORCO. The axes represent the indices along the ORCO primary sequence, along which predicted transmembrane helical segments (TMHs) are annotated as blue bars and predicted helical secondary structure as grey bars. Black dots represent ECs between pairs of residues; the representation is mirror-symmetric along the diagonal. The lines of ECs parallel and anti-parallel to the diagonal of the contact map are characteristic of the helix packing arrangements observed in alpha-helical transmembrane proteins¹. Three regions of high-density ECs within the N-terminal tail, extracellular loop 2 and intracellular loop 3 are highlighted by red dashed circles. The ORCO-specific insertion in intracellular loop 2 was excluded from statistical analysis and modelling (see Methods).

Supplementary Table 1 | Sources of OR protein sequences.

Species	# ORs	Reference
<i>Drosophila melanogaster</i>	60	2
<i>Drosophila simulans</i>	65	2
<i>Drosophila sechellia</i>	65	2
<i>Drosophila yakuba</i>	63	2
<i>Drosophila erecta</i>	58	2
<i>Drosophila ananassae</i>	71	2
<i>Drosophila pseudoobscura</i>	55	2
<i>Drosophila persimilis</i>	55	2
<i>Drosophila willistoni</i>	73	2
<i>Drosophila mojavensis</i>	52	2
<i>Drosophila virilis</i>	53	2
<i>Drosophila grimshawi</i>	83	2
<i>Drosophila eugracilis</i>	58	This study; Genbank
<i>Drosophila takahashii</i>	62	This study; Genbank
<i>Drosophila kikkawai</i>	70	This study; Genbank
<i>Drosophila bipectinata</i>	61	This study; Genbank
<i>Drosophila elegans</i>	62	This study; Genbank
<i>Drosophila biarmipes</i>	60	This study; Genbank
<i>Drosophila rhopaloa</i>	58	This study; Genbank
<i>Drosophila ficusphila</i>	65	This study; Genbank
<i>Drosophila miranda</i>	65	This study; Genbank ³
<i>Aedes aegypti</i>	131	4, 5
<i>Culex quinquefasciatus</i>	269	6
<i>Anopheles gambiae</i>	79	7
<i>Anopheles darlingi</i>	23	8
<i>Bombyx mori</i>	69	9
<i>Manduca sexta</i>	65	10, 11
<i>Heliothis viriscens</i>	18	12
<i>Heliothis subflexa</i>	5	13
<i>Helicoverpa spp.^a</i>	57	13; Genbank
<i>Epiphyas postvittana</i>	7	14
<i>Spodoptera littoralis</i>	37	15
<i>Ctenopseustis spp.^b</i>	7	16
<i>Planotortrix spp.^c</i>	10	16
<i>Cydia pomonella</i>	49	17
<i>Ostrinia spp.^d</i>	167	18-23; Genbank
<i>Danaus plexippus</i>	45	24
<i>Tribolium castaneum</i>	340	25, 26

<i>Megacyllene caryae</i>	57	²⁷
<i>Nasonia vitripennis</i>	301	²⁸
<i>Cotesia vestalis</i>	162	²⁹
<i>Pogonomyrmex barbatus</i>	399	³⁰
<i>Linepithema humile</i>	367	³¹
<i>Harpegnathos saltator</i>	377	³²
<i>Camponotus floridanus</i>	407	³²
<i>Solenopsis invicta</i>	297	³³
<i>Acromyrmex echinatior</i>	78	³⁴
<i>Apis mellifera</i>	174	³⁵
<i>Apis florea</i>	96	Genbank
<i>Bombus impatiens</i>	134	Genbank
<i>Bombus terrestris</i>	113	Genbank
<i>Megachile rotundata</i>	123	Genbank
<i>Acyrtosiphon pisum</i>	79	³⁶
<i>Pediculus humanus humanus</i>	10	³⁷
various insect ORCOs	41	³⁸

Footnotes:

^a *H. armigera*, *H. assulta*, *H. zea*

^b *C. herana*, *C. obliquana*

^c *P. excessana*, *P. notophaea*, *P. octo*

^d *O. furnacalis*, *O. latipennis*, *O. nubilalis*, *O. ovalipennis*, *O. palustralis*, *O. scapularis*, *O. zaguliaevi*, *O. zealis*

Supplementary Table 2 | Functional annotation of OR residues.

OR	a.a. position	Dme/OR85b equivalent	Dme/ORCO equivalent	Effect of mutation	Ref.
Dme/OR59b	V91	V77	F84	affects odour inhibition by 1-octen-3-ol and DEET	39
OfurOR3	T148	L138	S146	affects pheromone specificity	18
Dme/OR85b	F142	F142	W150	affects 2-heptanone specificity	40
Dme/OR85b	N143	N143	T151	affects 2-heptanone specificity	40
Dme/OR85b	C146-Y150	C146-Y150	T154-D158	affects 2-heptanone specificity	40
AgamOR15	A195	G193	V206	affects acetophenone/4-methylphenol response ratio	41
Dme/OR67d	C23	F11	A23	abolishes receptor function <i>in vivo</i>	42
BmorORCO	E171	L157	D165*	affects rectification index and reversal potential	43
BmorORCO	E329	R246	E343	affects rectification index and reversal potential	43
BmorORCO	D343	D260	D357	affects rectification index and reversal potential	43
BmorORCO	E422	V338	E436	affects rectification index and reversal potential	43
BmorORCO	Y464	F380	Y478	affects rectification index, reversal potential and cation selectivity	43
Dme/ORCO	T393-G399	K295-V301	T393-G399	reduces K ⁺ permeability; affects outward rectification	44
Dme/ORCO	C87	G80	C87	slightly reduces ORCO homomer function	45
Dme/ORCO	C228	V215	C228	reduces ORCO homomer function	45
Dme/ORCO	C409	C311	C409	increases ORCO homomer function	45
Dme/ORCO	C429	Q331	C429	increases ORCO homomer function; decreases ORCO/OR22a heteromer function	45
Dme/ORCO	C446	I348	C446	reduces ORCO homomer function	45
Dme/ORCO	C449	S351	C449	increases ORCO homomer function; decreases ORCO/OR22a heteromer function	45
Dme/ORCO	D466	S368	D466	affects receptor activity	46
BmorOR1	Y170	W151	S159	affects rectification index and reversal potential	43
BmorOR1	D226	D204	D217	affects rectification index and reversal potential	43
BmorOR1	D299	A257	A354	affects rectification index, reversal potential and cation selectivity	43
BmorOR1	E325	Q283	Q380	affects rectification index and reversal potential	43
BmorOR1	E356	Q315	N413	affects rectification index, reversal potential and cation selectivity	43
BmorOR1	D367	V326	E424	affects rectification index and reversal potential	43
BmorOR1	E375	Y334	Y432	affects rectification index and reversal potential	43
BmorOR1	D378	D337	S435	affects rectification index and reversal potential	43

* lower-confidence alignment

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